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ABSTRACT: There is growing evidence that speciation can occur between populations that are not geographically isolated. The emergence of assortative mating is believed to be critical to this process, but how assortative mating arises in diverging populations is poorly understood. The wolf spider genus *Schizocosa* has become a model system for studying mechanisms of assortative mating. We conducted a series of experiments to identify the factors that control mate pair formation in a *Schizocosa* population that includes both ornamented and nonornamented males. We show that the population also includes two previously unrecognized female phenotypes. One female phenotype mates mostly or exclusively with ornamented males, and the other mates mostly or exclusively with unornamented males. Assortative mating within these groups is maintained by differences in maturation time, microhabitat use, and female mate preference. We conclude that the population is not a single species, as previously believed, but rather an incipient species pair with multiple overlapping mechanisms of reproductive isolation. The identification of a new incipient species pair in the well-studied and rapidly speciating *Schizocosa* clade presents new opportunities for the study of speciation without geographic isolation.

Keywords: speciation, assortative mating, mate choice, allochrony, habitat choice, *Schizocosa*.

Introduction

Speciation is the process by which reproductive isolation evolves within and among populations (Coyne and Orr 2004). It has helped shape Earth's biodiversity (Wiens and Donoghue 2004) and can both create and fill ecological niches (Schluter 2000; Nosil 2012). For these reasons, speciation is of great interest to evolutionary biologists and ecologists alike, and understanding the mechanisms that underlie speciation has been a major goal of researchers since Darwin (1859). There are many examples of speciation in allopatry, where physical isolation prevents gene flow between diverging populations (Coyne and Orr 2004). There are fewer well-documented examples of speciation when gene flow is not interrupted by physical barriers (Coyne and Price 2000; Bolnick and Fitzpatrick 2007), and the mechanisms that promote speciation in such cases are not fully understood (Bolnick and Fitzpatrick 2007; Gavrilets 2014).

Assortative mating (i.e., the tendency to mate with individuals phenotypically or genetically similar to oneself) is believed to be critical to speciation without geographic isolation in sexually reproducing animals (Schluter 2000; Coyne and Orr 2004; Nosil 2012). By inhibiting mating between subpopulations, assortative mating allows the genotypes of those subpopulations to diverge. Assortative mating can arise when the members of diverging populations use their habitats differently in time or in space (Fitzpatrick et al. 2008). For example, different populations of Madeiran storm petrels (*Oceanodroma castro*) on the same island breed at different times, resulting in a nearly complete lack of gene flow between them (Friesen et al. 2007). In contrast, some flies in the genus *Rhagoletis* are active in the same habitat at the same time but breed on different host plants, causing assortative mating based on microhabitat use (Feder and Bush 1989; Feder et al. 1989; Dres and Mallet 2002). Differences in the use of time and space among subsets of animals in formerly randomly mating populations are believed to have been important in some speciation processes (e.g., Feder and Bush 1989; Friesen et al. 2007).

Mate preferences, whether learned or genetically determined, can also promote assortative mating. Mate prefer-

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ence learning can occur at different life-history stages, and which mate preferences are learned can depend on the social environment (reviewed in Hebets and Sullivan-Beckers 2010). For example, in some sticklebacks (e.g., *Gasterosteus aculeatus*) and cichlids (e.g., *Mbipia* spp., *Apistogramma cacatuoides*), females learn as juveniles to prefer mates that are genetically similar to themselves by observing the phenotypes of their parents (Verzijden et al. 2008; Kozak and Boughman 2009; Romer et al. 2014). In other species, such as the fruit flies *Drosophila persimilis* and *Drosophila melanogaster* and the Japanese quail *Coturnix coturnix japonica*, adults (often males) learn mate preferences from success or failure in previous mating attempts (Nash and Domjan 1991; Dukas 2008; Verzijden et al. 2015). In such cases, experienced males tend not to court females with phenotypes similar to those that have rejected them in the past, and mate preference learning by males can strengthen assortative mating that arises due to female mate preferences (Dukas 2008). In still other species, the ability to mate assortatively is genetic. For example, in the cricket frog *Acris crepitans*, females have genetic preferences for the courtship displays of males from their own populations (Ryan and Wilczynski 1988). Similarly, pied flycatchers (*Ficedula hypoleuca*) are able to identify and select conspecific mates even if they have been cross fostered by other species (Slagsvold et al. 2002; Slagsvold 2004).

Mate preferences can be based on any phenotype that differs among potential mates and that choosy individuals can perceive (Kilmer et al. 2017). Such phenotypes can include visual (e.g., coloration, patterning, ornament size, body size, movement), acoustic (e.g., song characteristics), and chemical (e.g., pheromone profiles) cues. Which phenotypes are used in mate choice and how strongly mate preferences are expressed can depend on the environment in which mates are evaluated (Miller and Svensson 2014). For example, three-spined sticklebacks (*G. aculeatus*) base their mate choice on visual cues (including courtship behavior; Candolin 1997) when the water is clear but on olfactory cues when the water is turbid, and mate choice patterns under these two conditions may not coincide (Heuschele et al. 2009). In green swordtails (*Xiphophorus helleri*), predator exposure influences female mate choice: females switch their mate preference from males with long swords to males with swords removed following exposure to predation on conspecific males with long swords (Johnson and Basolo 2003). Environmental conditions can also affect the strength of assortative mating. For example, brown trout (*Salmo trutta*) in experimental manipulations were more likely to choose mates from their own populations when mating occurred in highly variable flow regimes than in constant flow regimes (Gauthey et al. 2016).

The evidence presented above shows that assortative mating can arise from differences in reproductive timing (i.e., allochrony), microhabitat use, and learned or genetic mate preferences. Moreover, it is possible and even likely for multiple mechanisms to operate in the same system. In this article, we explored several potential mechanisms of assortative mating in *Schizocosa* wolf spiders. In particular, we focused on an intriguing population with two male morphotypes that exhibit different phenotypes and courtship behaviors.

The Schizocosa *System*

Schizocosa (family Lycosidae) is a genus of cursorial wolf spiders. The genus includes ~58 species and is globally distributed (World Spider Catalog 2017), but the monophyly of the genus—particularly of the species from outside North America—has been questioned (Stratton 2005). Most *Schizocosa* are univoltine. The exact phenology varies among species and populations, but in many species mated females produce egg sacs in late spring or early summer and then guard or carry their egg sacs attached to their spinnerets (Dondale 1977). Eggs hatch and juveniles emerge in mid- to late summer, and offspring are carried on the female's abdomen for up to 2 weeks before dispersing (Montgomery 1903; Dondale 1977). Adult males die earlier than females, but adults of both sexes die by late summer, and offspring overwinter as juveniles (Montgomery 1903; Dondale 1977; Uetz and Denterlein 1979). In the spring, juveniles mature and mating occurs (Dondale 1977). The mating behavior of several North American *Schizocosa* species has been well studied and is variable and often elaborate (Montgomery 1903; Uetz and Denterlein 1979; Stratton and Uetz 1981; Stratton 1991, 1997; Hebets et al. 1996; Scheffer et al. 1996; Hebets 2003; Hebets and Vink 2007; Vaccaro et al. 2010; Hebets et al. 2013; Fowler-Finn et al. 2015). As a result, the genus has become a model for studying courtship and mate selection in invertebrates (Herberstein and Hebets 2013).

In the Ohio Valley of the United States, some *Schizocosa* populations include sexually mature males that possess either (i) tufts or brushes of dark hair on the tibiae of their forelegs or (ii) no tufts or pigmentation on their foreleg tibiae (Dondale and Redner 1978). Tufts appear at sexual maturity and are believed to be sexual ornaments (Uetz and Denterlein 1979; Uetz 2000; Uetz and Norton 2007). Each adult male morphotype produces a distinct stereotyped courtship display (Stratton and Uetz 1981, 1986), but the two male morphotypes are not distinguishable by genital morphology, body size, or body color (Stratton and Uetz 1981). Females in these populations have no tufts and no distinguishable morphotypes (Stratton and Uetz 1981). Nonetheless, Uetz and Dondale (1979) showed that each male phenotype is associated with a cryptic population of females, and the two male-female population pairs are re-

productively isolated. Thus, despite the morphological similarity between females, the populations have been classified as separate species: the ornamented species is *S. ocreata*, and the nonornamented species is *S. rovneri* (Uetz and Dondale 1979). Studies in the 1970s and 1980s demonstrated that reproductive isolation between the species is maintained by strong female preferences for conspecific mating displays (Uetz and Denterlein 1979; Stratton and Uetz 1981, 1986), and recent molecular work has confirmed that *S. ocreata* and *S. rovneri* are genetically distinct (Fowler-Finn et al. 2015). Thus, the species pair is an example of behavioral isolation.

Decades after the work that established *S. ocreata* and *S. rovneri* as separate species, a *Schizocosa* population in Oxford, Mississippi, was discovered that includes both ornamented (cf. *S. ocreata*) and nonornamented (cf. *S. rovneri*) males (Hebets and Vink 2007). Ornamented and nonornamented males in this population were subsequently found to be genetically distinct from *S. ocreata* and *S. rovneri* but not from each other (Fowler-Finn et al. 2015). The population has been hypothesized to be panmictic with the competing morphotypes maintained by frequency-dependent natural selection (Deng et al. 2014). If reproductive isolation exists between the morphotypes in this population, the lack of genetic divergence at rapidly evolving microsatellite markers suggests that it is either less complete or more recent than the reproductive isolation between *S. ocreata* and *S. rovneri*.

In both the Ohio Valley *Schizocosa* population and the Oxford *Schizocosa* population, ornamented and nonornamented males differ in their phenology and habitat use. In the Ohio Valley, ornamented *S. ocreata* males mature 2–3 weeks earlier than nonornamented *S. rovneri* males (Uetz and Denterlein 1979). The two species can be found syntopically, but *S. rovneri* is more common in floodplains and *S. ocreata* is more common in upland habitats (Uetz and Denterlein 1979; Stratton and Uetz 1986). There is preliminary evidence for partial allochrony of male morphotypes in the Oxford population, but the direction is opposite that in the Ohio Valley: ornamented males in the Oxford population mature ~7 days later than nonornamented males (Hebets and Vink 2007). In the Oxford population, ornamented males are found more frequently on rocky substrate, and nonornamented males are found more frequently on leaf litter (Deng et al. 2014). This difference may be due to habitat choice rather than differential mortality, as the two male morphotypes show equal survival on either substrate (Fowler-Finn and Hebets 2011*b*). While male morphotypes in the Oxford population differ in maturation time and microhabitat use, no studies have yet explored how these differences influence mate pair formation.

Female mate preferences are also known to affect mate pair formation in the Ohio Valley and Oxford *Schizocosa* populations. In both populations, females have genetic preferences for the courtship displays of particular male morphotypes. Studies that have attempted to mate individuals of known parentage have found that females mate preferentially with males that match their fathers' phenotypes (Stratton and Uetz 1986; Fowler-Finn et al. 2015). In addition to genetic preferences, roles for mate choice learning have been suggested in these populations (Hebets 2003; Hebets and Vink 2007; Fowler-Finn et al. 2015; Stoffer and Uetz 2016*a*, 2016*b*). Within *S. ocreata*, females can learn to prefer different male ornament sizes based on previous courtship experience (Stoffer and Uetz 2016*a*, 2016*b*), but courtship experience does not influence preference for conspecific mates in either *S. ocreata* or *S. rovneri* (Rutledge and Uetz 2014). In the Oxford population, researchers have suggested that both juvenile experience and mate choice copying may help to shape female mate preferences for particular male phenotypes (Hebets and Vink 2007; Fowler-Finn et al. 2015).

Research Objectives

Our goal in this study was to identify the factors that contribute to mate pair formation in the Oxford *Schizocosa* population. We analyzed a data set that was collected more than 12 years ago for this purpose, and we identified the factors that predict (i) male and female sexual maturation times, (ii) whether males court a given female, (iii) whether females mate when courted, and (iv) which male morphotype females choose. Our results suggest that, in addition to its two male morphotypes, the Oxford *Schizocosa* population includes two female maturation groups. Each female maturation group mates preferentially with a particular male morphotype, and these male-female pairs comprise incipient species. Our results show that multiple coinciding mechanisms can combine to promote reproductive isolation even very early in the speciation process, and this will help to make the Oxford *Schizocosa* population a model system for studying the evolution of reproductive isolation.

Methods

Spider Collection and Rearing

We collected 460 immature *Schizocosa* near the Ole Miss greenhouse, Oxford, Mississippi, on the nights of March 19 and 21 and during the day on March 21, 2005. We collected spiders from rock litter adjacent to the greenhouse building and from leaf litter ~100 m from the building. We recorded the substratum type (rock or leaf litter) from which each spider was collected. The site has been repurposed since 2005,

and the exact locations where we collected spiders are no longer available.

Rearing Environment

Exposure to other spiders during the juvenile stage can affect mate preference in *Schizocosa* (Hebets 2003; Stoffer and Uetz 2016*a*) and maturation rate in other spiders (Kasumovic and Andrade 2006). Given the previously observed differences in maturation rate between ornamented and nonornamented males in the Oxford population (Hebets and Vink 2007), we wanted to explore the influence of juvenile exposure to other spiders on mate choice and maturation rate in this population. To do this, we manipulated the environment to simulate different exposure rates that spiders might encounter in the wild. We assigned spiders to one of three treatments: (i) central ($N = 64$), (ii) peripheral ($N = 256$), or (iii) isolated ($N = 140$). Central and peripheral spiders, set up as in figure 1, were exposed to visual and chemical cues from other spiders, while isolated spiders were not. Each central spider was placed in a 6 cm × 6 cm × 8 cm plastic box (AMAC Plastic Products, Petaluma, CA). Next to each side of each central spider's box, we placed a similar box containing a peripheral spider. The walls of the boxes were clear, so visual signals could be sent and received between adjacent spiders. To provide chemical cues, we used pieces of flat wooden craft sticks to collect deposited silk and excreta. We cut the craft sticks into equal-sized pieces and placed four pieces at the bottom of each individual's box. After 3–4 days of collecting chemical cues, one piece of stick was removed from each peripheral spider's box and placed in the central spider's box, and each of the four pieces from the central spider's box was removed and placed in one of the peripheral spiders' boxes. The silk-laden pieces remained in the boxes for ~4 days. We repeated this process with new wooden craft sticks each week for the duration of the experiment. Thus, central spiders received constant visual and periodic chemical cues from four peripheral spiders, while peripheral spiders received constant visual and periodic chemical cues from one central spider. Isolated individuals were placed in boxes of the same size but were visually isolated from other spiders. Pieces of wooden craft sticks were placed at the bottom of each isolated spider's box and were replaced with new pieces every time the sticks were replaced for central and peripheral spiders. This ensured that disturbance was similar across treatments but did not expose isolated spiders to the deposited silk or excreta of other spiders. Some spiders use airborne pheromones to communicate mating status (e.g., Watson 1986). Because all spiders were reared in the same laboratory space we cannot guarantee that the spiders we called isolated were not exposed to airborne pheromones, although no such pheromones have been shown to exist in

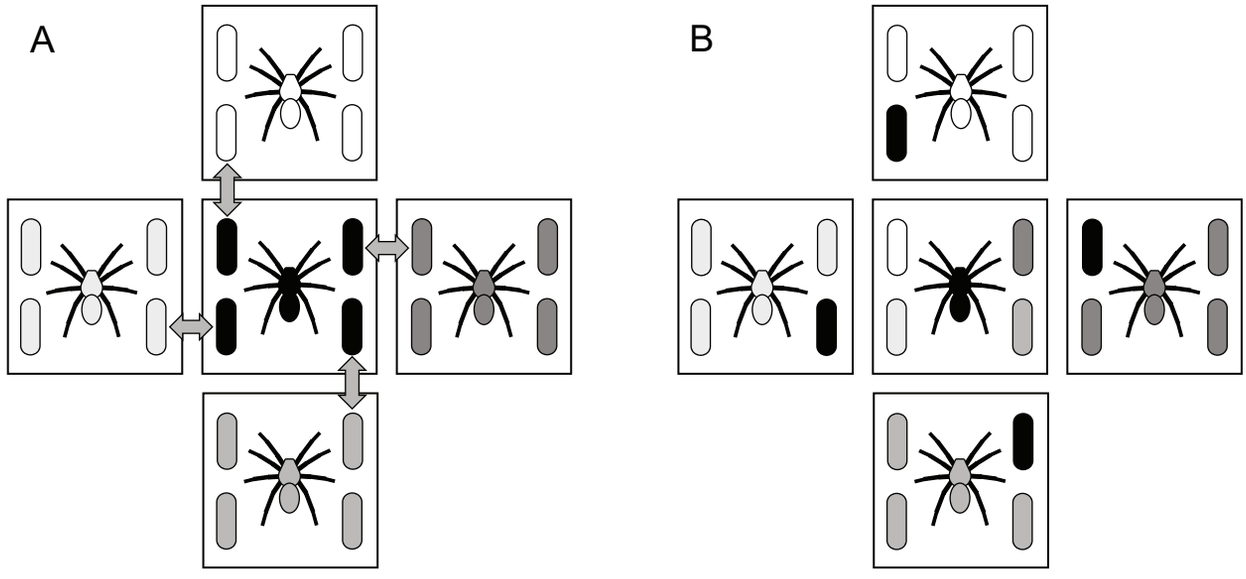

Figure 1: Rearing arrangement. Spiders were raised in 6 cm × 6 cm × 8 cm clear plastic boxes. To expose spiders to conspecifics, boxes were arranged in quintuples as shown (64 quintuples, 320 spiders). Every 7 days, we placed four pieces of clean flat wooden craft sticks on the bottom of each box (*A*). After 3–4 days, craft stick pieces were exchanged between adjacent boxes (*B*). Thus, peripheral spiders were exposed to constant visual and periodic chemical signals from one central spider, and central spiders were exposed to constant visual and periodic chemical cues from four different peripheral spiders. Isolated spiders (140 spiders, not pictured) were reared in similar boxes but were visually isolated and experienced no exchange of craft sticks with other spiders.

Schizocosa. We checked spiders at least every other day to monitor molting and maturation times. Spiders were maintained in their treatments without interruption until sexual maturity and then throughout the mate choice experiments. We observed courtship displays by males in the rearing chambers, but we made no attempt to count or record these. However, given these observations, we believe females that were reared next to males are likely to have encountered courtship displays.

In preliminary analyses, we found no difference in the maturation time or mating behavior of central and peripheral spiders (see fig. S1; figs. S1, A1 are available online). Therefore, in subsequent analyses we combined these treatment groups, leaving us with two exposure treatments: exposed and isolated.

Mate Choice Trials

Each mate choice trial took place in a circular plastic arena (diameter, 20.3 cm; height, 7.6 cm; Pioneer Plastics, Dixon, KY). Spiders in the Oxford population can be found on rocky substrates or on leaf litter and are active at different times of the day, thus encountering different light conditions. Therefore, we wanted to test the influence of the physical environment on mate choice. We manipulated both the substrate and the light environment in mating trials in a fully crossed 2 × 2 design. Arenas were filled with a single substrate (i.e., either rock litter or leaf litter collected from the Oxford site) to a depth of ~5 cm, and trials were conducted either in the light or in the dark. Light trials were conducted on a benchtop underneath a skylight that provided natural light, and dark trials were conducted behind a black curtain that blocked all light and were observed with an infrared camera. No attempt was made to standardize natural light conditions in response to time of day or weather.

Prior to the start of each trial, one female was placed in the mating arena with the substrate and light environment to which she had been randomly assigned, and she was allowed to acclimate for 5 min. Then one ornamented and one nonornamented male were simultaneously placed on opposite sides of the arena, as far from the female and from each other as possible. We allowed all three spiders to interact freely for 45 min, and we recorded (i) whether each male courted, (ii) which male courted first, (iii) whether the female mated, and (iv) which male she mated with if she mated. Each female participated in only one mating trial, but due to a shortage of available males some males participated in two trials. In 148 mating trials with two males per trial, 156 males were used once and 70 males were used twice. We allowed at least 2 days between mating trials for the same male, and no male was paired with the same competing male more than once. When male identity was treated as a random effect in subsequent analyses, it did not affect our response variables (likelihood ratio tests, $P > .99$). We tested for main effects of previous courtship or mating experience in each analysis.

Statistical Analyses

There are many factors that might influence spider maturation rates and mating decisions (e.g., male morphotype [Hebets and Vink 2007] and spider age, mass, and juvenile experience [Uetz and Norton 2007]; see the figures in "Results" for full lists of the factors we studied and tables S1–S5 [tables S1–S6, A1 are available online] for the numbers of spiders in each class when factors were categorical; full data sets have been deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.qc6176k [Gilman et al. 2018]). Therefore, we analyzed our data using a factor selection approach. Factor selection aims to identify strong relationships between possible predictors and response variables rather than to test specific a priori hypotheses. The results offer insight into the behavior of complex systems and provide testable hypotheses for further study (Burnham and Anderson 2002). There is no consensus on the best method of factor selection. Therefore, we performed both information theoretic (Burnham and Anderson 2002, 2004) and lasso (least angle shrinkage and selection operator; Tibshirani 1996) analyses. Either method can produce false positives, but if both methods identify the same predictor as important, then we can have more confidence that the predictor is meaningful than if it were identified by only one method. We used our analyses to identify predictors associated with each of four biological responses: (i) the number of days from collection to maturity, (ii) whether a male courted in a mating trial, (iii) whether a female mated in a mating trial, and (iv) if a female mated, whether she mated with the ornamented or nonornamented male in her trial.

To implement the information theoretic approach, we fit linear regressions (for days to maturity) or logistic regressions (for other response variables) that included every possible combination of the predictors we studied for each response variable. We calculated Akaike's information criterion corrected for small sample size for each fitted model, and we found each model's Akaike weight. For each predictor, we summed the weights of all the models in which that predictor appears to obtain the summed weight for that predictor. Summed weight measures the probability that the predictor appears in the best model, given the set of all models studied. To estimate the effect size of each predictor on the response variable, we averaged its effect size across all models in which it appears, with each model weighted according to its Akaike weight. Finally, we calculated the confidence distribution for the effect size of each predictor in each model in which it appears (Xie and Singh 2013). We weighted these distributions by the Akaike weights of the models and

summed across all models in which the predictor appears to obtain the unconditional confidence distribution for the effect size of that predictor.

We compared the results of our information theoretic analysis to those of a lasso analysis. Lasso finds the model that best describes the data using potentially all predictors, subject to the constraint that the sum of the scaled effect sizes across all predictors is less than a tuning parameter *s*. We used cross validation to find the value of *s* that best describes the data without overfitting. Predictors that have nonzero effect sizes in this model are interpreted to have an effect on the response variable. We conducted the lasso analysis in R using the packages glmnet (Friedman et al. 2010) and hierNet (Bien et al. 2013).

For days to maturity, the number of biologically plausible predictors is small, and we considered all pairwise interactions between first-order predictors in our analyses. Our results are qualitatively unchanged if we exclude interactions from the analyses. For other analyses, the number of plausible predictors is large, and the number of possible pairwise interactions exceeds the number of observations in the data set. When the total number of predictors and interactions approaches or exceeds the number of observations, factor selection tends to identify spurious predictors (Freedman 1983; Anderson and Burnham 2002). Therefore, we focused on first-order predictors and excluded interactions in these analyses.

The residuals of the best-fit model for days to maturity in females (but not in males) are bimodally distributed. We used model-based clustering, implemented in R with the package mclust (Fraley and Raftery 2002), to find the maximum likelihood distributions of (i) days to female maturity and (ii) the residuals of days to maturity fitted against female origin. Then we calculated the probability that each female in the data set belonged to the early- or late-maturing group on the basis of her observed days to maturity. Having done this, we wanted to know whether females collected from leaf litter were more likely to belong to the early-maturing group (as is the case for males). Because the assignment of females to maturation groups is probabilistic rather than absolute, we could not use a χ^2 test to look for an association between female origin and maturation group. Therefore, we used a Monte Carlo analysis. We maintained the group assignment probabilities and randomized the observed collection substrates among all females in the data set, and we calculated the mean probability with which females from leaf litter belonged to the early-maturing group. We repeated this process 10^5 times to obtain a null distribution of association strengths that we could have obtained by chance alone. We compared the observed data to this null distribution to assess the significance of the relationship.

Our analysis of male courtship includes one extreme outlier. One male did not court, even though all plausible models predict that his probability of courting was greater than 0.9999. Excluding this outlier from the analysis does not change our qualitative results. We present results with the outlier included. Results with the outlier excluded are shown in figure S2.

Estimating the Strength of Prezygotic Reproductive Isolation

Analysis of our empirical data suggests that the Oxford *Schizocosa* population comprises two maturation groups that are partially reproductively isolated by a combination of allochrony, habitat use, and female mate choice (see "Results"). To study the strength of prezygotic reproductive isolation arising from these mechanisms, we constructed a dynamical systems model that simulates maturation, habitat use, and mating in the wild population for one generation (appendix, available online). We used this model to estimate the proportion of females from each maturation group that accept mates from the other maturation group.

Results

Maturation Time

We recorded days to maturity for 192 male and 193 female spiders. The predictors of male maturation time are shown in rows 1–6 of figure 2. Nonornamented males matured 8.9 days earlier than ornamented males. There was no evidence that any other predictor affected male maturation time, but males collected from rocks were more likely than males collected from leaf litter to belong to the ornamented (i.e., late-maturing) group (73% vs. 44%; $\chi^2 = 15.08$, df $= 1$, $P = .0001$).

The predictors of female maturation time are shown in rows 7–9 of figure 2. There was a weak trend toward later maturation in females collected from rocks. Female maturation times were bimodally distributed (fig. 3). The earlier female mode matured at ~30 days, in synchrony with the nonornamented males, and the later mode matured at ~49 days, nearly 10 days after the ornamented males. Females collected from leaf litter were more likely than females collected from rocks to belong to the early-maturing group (10^5 Monte Carlo simulations, $P = .0392$), but female origin does not explain the bimodality in maturation times (table S6).

Male Courtship

We analyzed data from 148 mating trials. The predictors of male courtship are shown in figure 4. In 86 of 87 trials where one male courted, the other male also courted. In 73 of 74 trials where males were presented to females that

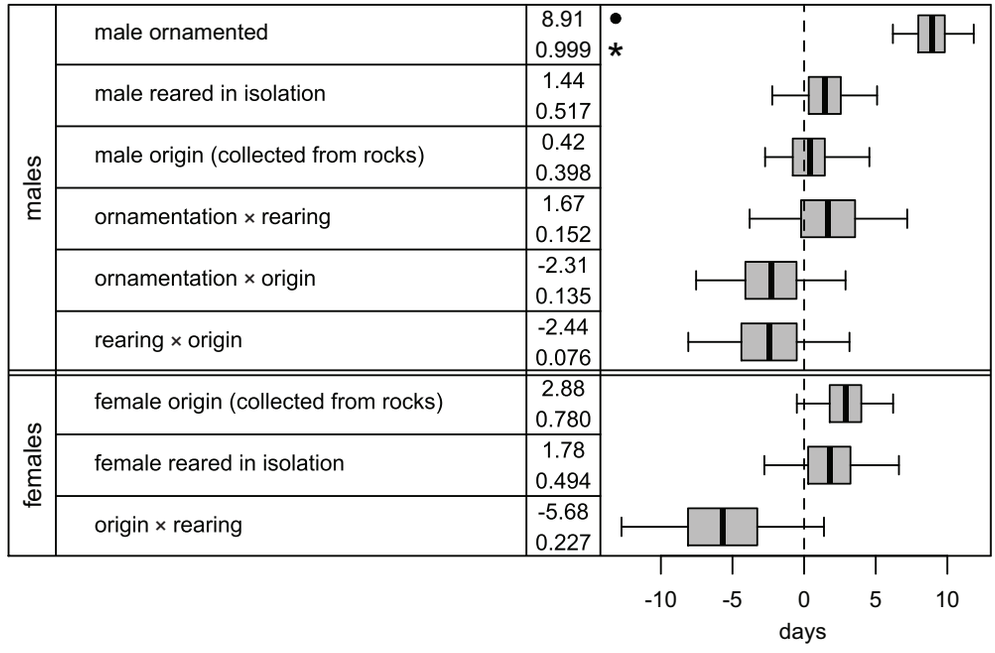

Figure 2: Predictors of days to maturity for males (rows 1–6) and females (rows 7–9). In the middle cell of each row, the top number is the effect size (days), and the bottom number is the summed weight of the predictor across all fitted models. Boxplots show the 50% (boxes) and 95% (whiskers) confidence intervals around the effect sizes. Filled circles indicate that zero falls outside the 99.9% confidence interval for the effect size of the predictor. Asterisks indicate that the predictor was identified as meaningful by the lasso analysis.

had been reared in isolation, both males courted. Thus, courtship by a competing male and the juvenile social environment of the female were strong predictors of male courtship. Males were more likely to court females that had more recently reached sexual maturity (1.1× d^{-1} since maturity) and females collected from rocks (4.6×). Ornamented males were 3.1 times more likely than nonornamented males to court first. Some males were used in multiple mating trials. Our lasso analysis suggests that males were more likely to court in their first trial than in subsequent trials if trials were illuminated and if trials were conducted on leaf litter substrate. The information theoretic analysis provides only weak support for the effects of prior trials, illumination, and substrate on courtship probability.

Female Mating

Females mated in 80 of 148 mating trials (i.e., 54%). The predictors of whether a female mated are shown in figure 5. The best predictor of whether a female mated was whether she was courted: females that were courted were 67 times more likely to mate than females that were not courted. Older and larger females were more likely to mate than younger and smaller females (1.2× d^{-1} since capture and 1.02× mg^{-1}, respectively). The information theoretic analysis, but not the lasso analysis, suggests that females were more likely to mate if there was at least one young male in the trial (0.90× d^{-1} of younger male age) and were more likely to mate if they had been reared with exposure to other spiders (7.4×). The lasso analysis suggests that females were more likely to mate in trials conducted on leaf litter, if they were collected from rocks, or if they had been exposed to males as juveniles.

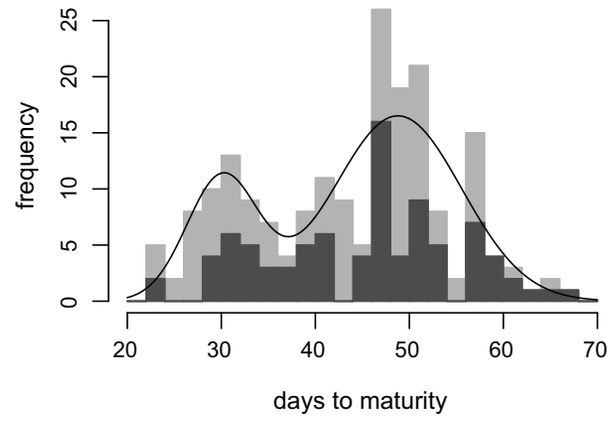

Figure 3: Distribution of days to maturity for females collected from leaf litter (light gray bars) and rocks (dark gray bars). The black line shows the maximum likelihood bimodal distribution of the data.

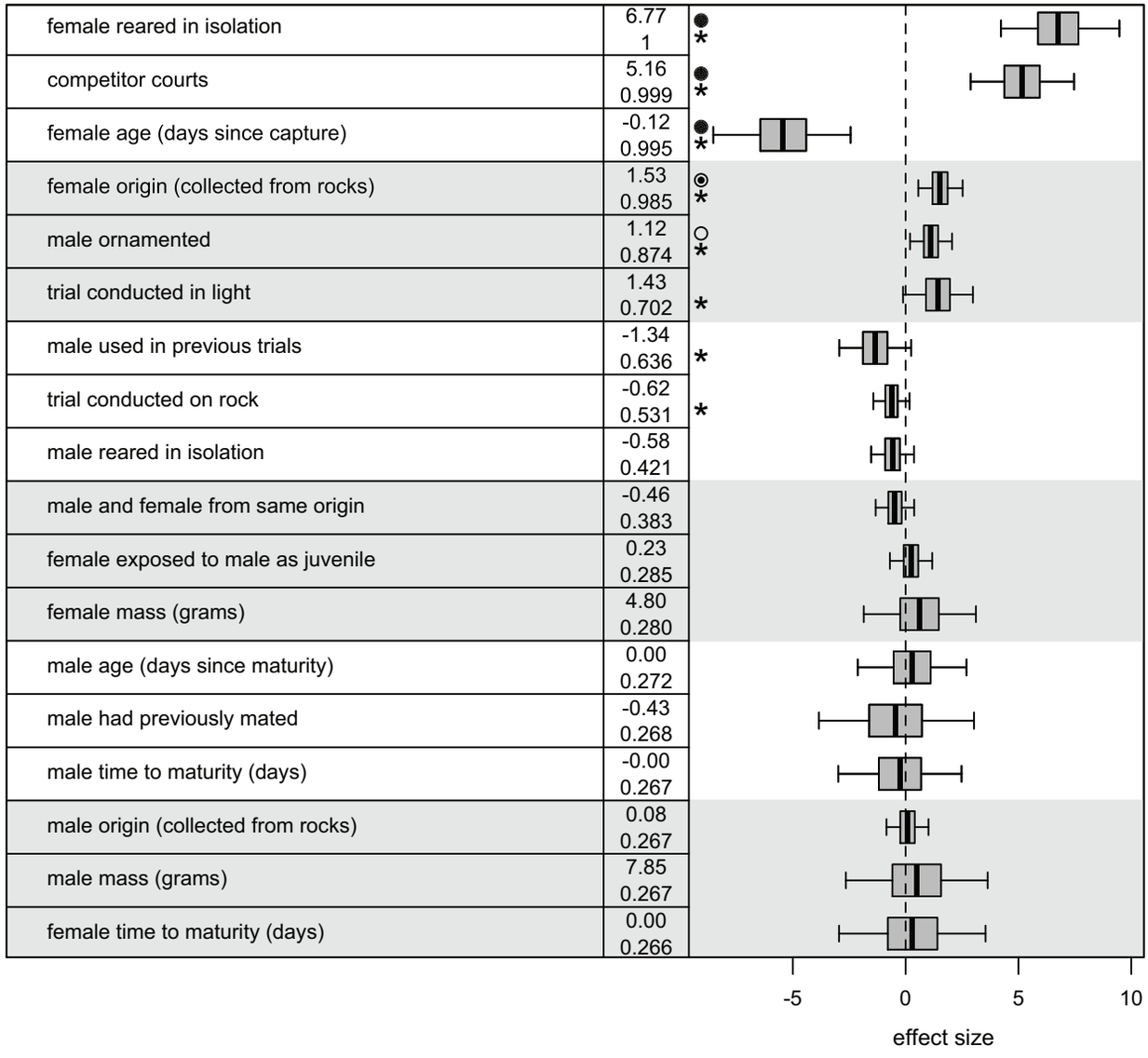

Figure 4: Predictors of male courtship. In the middle cell of each row, the top number is the effect size of the predictor on the log odds ratio of male courtship, and the bottom number is the summed weight of the predictor across all fitted models. Boxplots show the 50% (boxes) and 95% (whiskers) confidence intervals around the effect sizes. Effect sizes and confidence intervals in the boxplots are scaled to the maximum difference in the observed data for the associated predictor. For example, female mass in mating trials ranged from 0.050 to 0.172 g. Thus, the boxplot shows the effect of 0.122 g of female mass on the odds of male courtship. Scaling in this way does not change our confidence in the effects of the predictors but shows the relative importance of each predictor for male courtship given the variability in that predictor in the data. Filled circles, bull's-eyes, and open circles indicate that zero falls outside the 99.9%, 99%, and 95% confidence intervals, respectively, for the effect size of the predictor. Asterisks indicate that the predictor was identified as meaningful by the lasso analysis.

Although courtship was a strong predictor of female mating, 19 of 61 females that were not courted mated. In these cases, the absence of courtship was offset by predictors including age and mass. Among females that were not courted, those that mated were older (77.3 vs. 68.6 d since capture; Welch's t-test, $P = 1.29 \times 10^{-5}$) and larger (0.108 vs. 0.082 g; Welch's t-test, $P = 9.85 \times 10^{-4}$) than those that did not mate. Eighteen of 19 matings without courtship were achieved by ornamented males.

Female Mate Choice

Fifty-nine of 80 females (i.e., 74%) that mated did so with the ornamented male. The predictors of whether a female

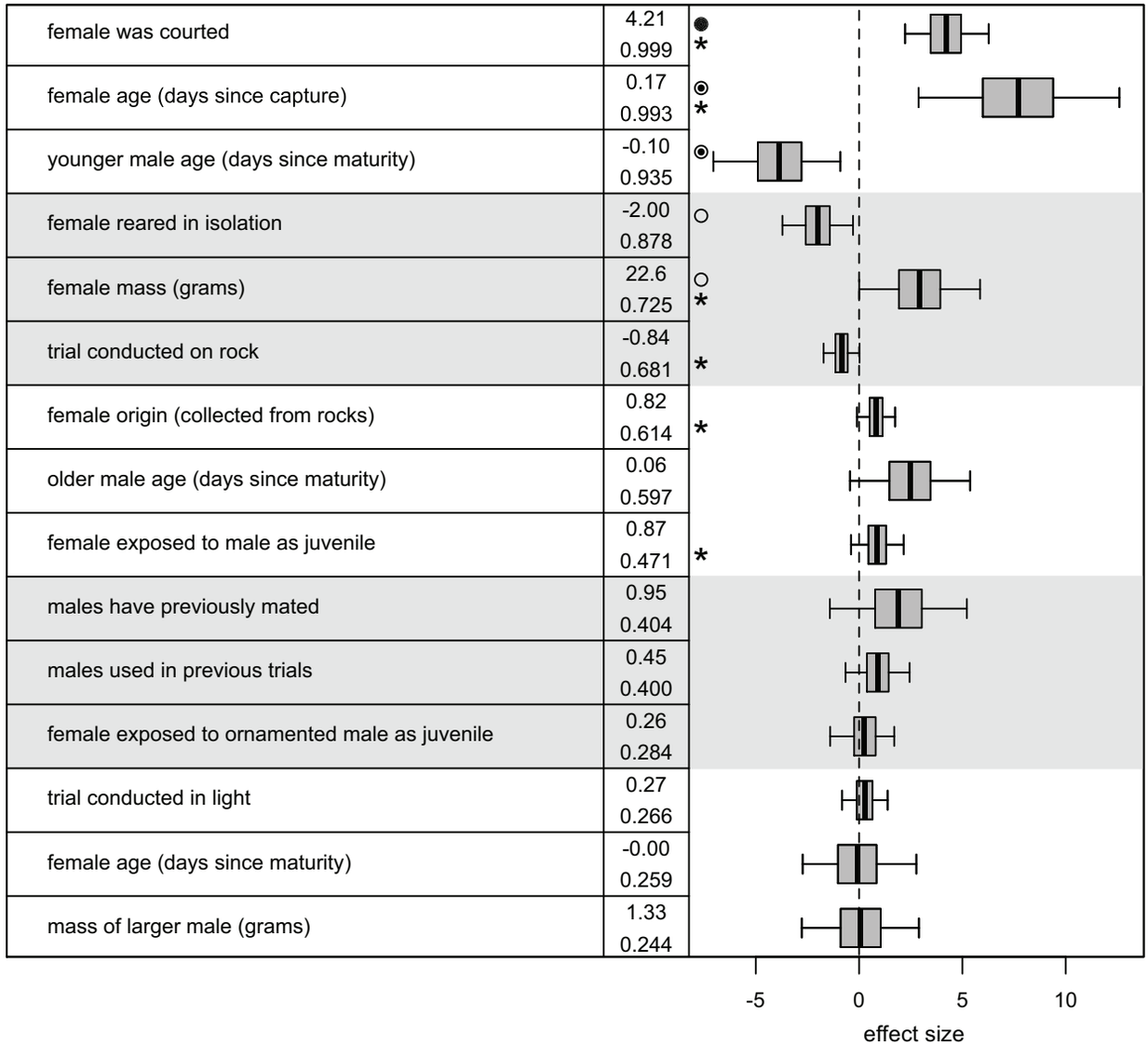

Figure 5: Predictors of whether a female mates. In the middle cell of each row, the top number is the effect size of the predictor on the log odds ratio of female mating, and the bottom number is the summed weight of the predictor across all fitted models. Boxplots show the 50% (boxes) and 95% (whiskers) confidence intervals around the effect sizes (scaled as in fig. 4). Filled circles, bull's-eyes, and open circles indicate that zero falls outside the 99.9%, 99%, and 95% confidence intervals, respectively, for the effect size of the predictor. Asterisks indicate that the predictor was identified as meaningful by the lasso analysis.

chose the ornamented or nonornamented male are shown in figure 6. Females assigned by cluster analysis to the late-maturing group were 14.9 times more likely than those assigned to the early-maturing group to choose ornamented males. The information theoretic analysis suggests that females were more likely to choose males that began to court first (6.0×) and males collected from the same origin as the female (5.0×) and that in the absence of courtship, females were more likely to mate with ornamented males (33×).

Strength of Prezygotic Reproductive Isolation

The above analysis suggests that the Oxford *Schizocosa* population comprises two maturation groups. One group includes early-maturing females and nonornamented males, and the other includes late-maturing females and ornamented males. These groups have different habitat preferences, and females prefer to mate with males from their own groups. On the basis of our empirical data, we estimate that the proportion of females from each maturation group

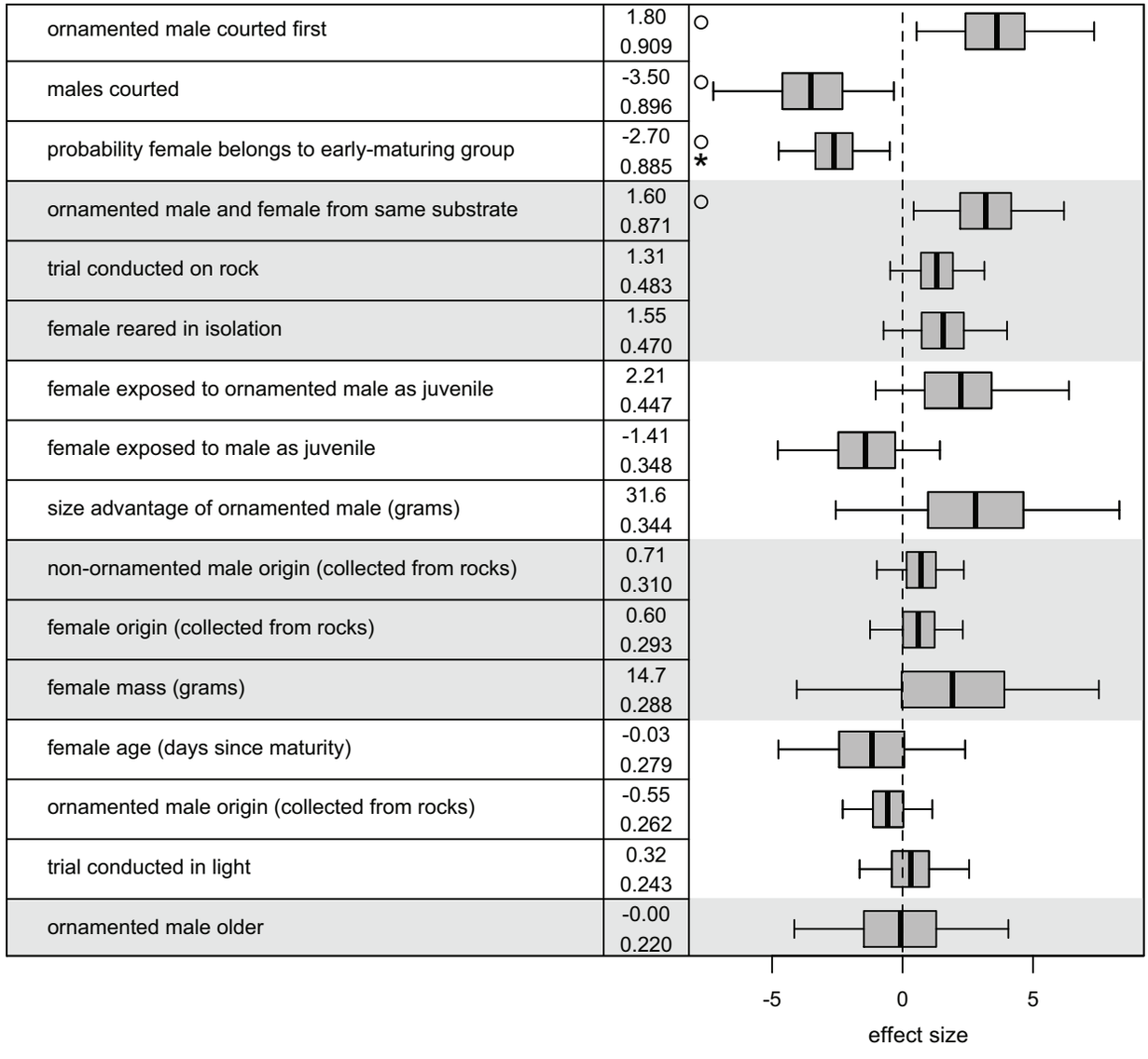

Figure 6: Predictors of whether a female chooses an ornamented male. In the middle cell of each row, the top number is the effect size of the predictor on the log odds ratio of the female choosing an ornamented male, and the bottom number is the summed weight of the predictor across all fitted models. Boxplots show the 50% (boxes) and 95% (whiskers) confidence intervals around the effect sizes (scaled as in fig. 4). Open circles indicate that zero falls outside the 95% confidence interval for the effect size of the predictor. Asterisks indicate that the predictor was identified as meaningful by the lasso analysis.

choosing mates from the opposite group in the Oxford population is ~0.07–0.18 (see the appendix). This is consistent with the low level of genetic divergence that has been observed between the male morphotypes.

Discussion

We analyzed data from laboratory rearing and mating experiments on *Schizocosa* wolf spiders collected from a polymorphic population in Oxford, Mississippi. We found that early-maturing females were disproportionately likely to choose mates of the early-maturing nonornamented male morphotype, and both early-maturing females and nonornamented males were likely to be found on leaf litter. Late-maturing females were more likely to choose mates of the late-maturing ornamented male morphotype, and both late-maturing females and ornamented males were likely to be found on rocky substrate. Taken together, these patterns suggest that the Oxford population is not a single freely interbreeding population, as previously assumed

(Hebets and Vink 2007; Deng et al. 2014; Fowler-Finn et al. 2015), but instead comprises two groups with partial reproductive isolation maintained by a combination of allochrony, habitat use, and female mate choice.

The maturation times and habitat use of males in our study are consistent with previous results. Hebets and Vink (2007) found that nonornamented males in the Oxford population matured earlier than ornamented males, and Deng et al. (2014) reported that nonornamented males were more likely to be found on leaf litter. However, our study is the first to show that females in this population also form two maturation groups, and we provide the first evidence that mate preference and habitat use by females are correlated with maturation time. The association of early-maturing females with leaf litter and late-maturing females with rocky substrate could be in part an effect of early juvenile habitat use in the field, as local prey availability and microclimate can affect maturation rates (Stratton 1984; Uetz et al. 2002; Rundus et al. 2010) and can vary across habitat types at small spatial scales (Widenfalk et al. 2016). However, origin does not fully explain the bimodality in maturation time for females in our study, and exposure to other spiders did not influence maturation times. Thus, differences in female maturation time must be due in part to inherent differences between the two female types. Because we collected spiders over a short period (i.e., 3 days) when only juveniles were present, we cannot confirm that maturation times in the wild match those we recorded in the laboratory. Future work that measures changes in male phenotype densities and female mate preferences in the wild population over the course of a full mating season would help to confirm our results.

The social environment influenced the probability of male courtship in two ways. First, males almost always courted when the competing male in their trial courted. This pattern could result from each male monitoring the behavior of the other (Clark et al. 2012, 2015). This would not require that males recognize each other's behavior as courtship but simply that courtship by one male draws the attention of the other to the presence of the female. Alternatively, the consistency of courtship behavior between males in the same trial could result from female receptivity signals (e.g., a behavior or pheromone; Sullivan-Beckers and Hebets 2014; Bell and Roberts 2017) that are perceived by both males. Behavioral and chemical receptivity signals can elicit or prolong male courtship in other *Schizocosa* species (Roberts and Uetz 2005; Vaccaro et al. 2010). Additionally, males were more likely to court females that lacked prior social experience with conspecifics. This observation is intriguing because it suggests that female receptivity signals might vary with social experience. Females might, for example, increase the production of a receptivity signal when perceived mate availability is low (Kokko and Monaghan 2001). Perceived mate availability is known to affect female mating behavior in some *Schizocosa* species (Stoffer and Uetz 2015*a*, 2015*b*) and female responsiveness in other taxa (Fowler-Finn and Rodríguez 2012*a*, 2012*b*). The density of male spiders at our study site was high during the sampling period, but the ability to moderate receptivity may be important for females in marginal habitats or in years when unfavorable conditions reduce the density of available mates.

Males were more likely to court some types of females than others. In particular, males courted younger females more often than older females. In the field, younger females are less likely to have mated previously, and males may use a female's age as a surrogate for assessing virgin status (Gaskett 2007). Younger females may also produce more or different sex pheromones (Uhl and Elias 2011) and so attract more male courtship. These two hypotheses are not mutually exclusive. Males were also more likely to court females collected from rocky substrate. The reason for this is unknown. However, we found weak evidence that females collected from rocky substrate were more likely to mate. It is possible that males are able to perceive increased female receptivity and increase their probability of courtship in response.

We found weak evidence that the physical signaling environment (i.e., substrate type and light conditions) also influenced whether males courted. Consistent with previous studies (Taylor et al. 2005; Gordon and Uetz 2011), males were more likely to court in illuminated trials and on leaf litter. This is not surprising, as both light and leaf litter facilitate signal transmission: visual signals cannot be perceived in the dark, and leaf litter is a good transmitter of vibratory sexual signals in *Schizocosa* (Elias et al. 2010).

The probability of mating for females also depended on social factors and on the physical environment. Not surprisingly, females that were courted were more likely to mate. What is surprising, however, is that females mated in 19 of 61 trials in which they were not courted. Eighteen of these 19 matings were with ornamented males. Attempted copulations of unreceptive females have been documented in our study population (Hebets and Vink 2007) and in other wolf spiders (Johns et al. 2009; Wilgers et al. 2009), but such a high frequency of copulations without courtship has not been observed previously. Copulation without courtship may be a strategy used by sexually aggressive ornamented males when multiple males compete for the same female. The density of the Oxford population can be as great as 300 individuals per square meter (Fowler-Finn and Hebets 2011*b*), so males may often compete directly for females in the field. It is also possible that females in these trials exhibited receptivity signals without being courted. Females that mated without courtship were older and larger than females that did not mate, and both age

and size correlate positively with receptivity to mating. Additional studies are needed to replicate and explain this result.

In addition to whether a female was courted, female mating probability depended on several other factors. Older females were more likely to mate than younger females. This is consistent with theory suggesting that choosiness should decrease when females expect fewer future mating opportunities (Moore and Moore 2001). Our information theoretic approach suggests that females were more likely to mate if they were exposed to other spiders as juveniles, and the lasso approach suggests that they were more likely to mate if they were exposed to males as juveniles. Because females exposed to males were necessarily exposed to other spiders, these predictors are correlated (Pearson's correlation coefficient $\rho = 0.425$), and we cannot be confident about which predictor affects female mating. However, it seems likely that either previous exposure to other spiders or previous exposure to males increases female mating probability, independent of the role played by exposure status in inducing male courtship. Finally, we found weak evidence that females were more likely to mate in trials on leaf litter than on rocks. Again, this may not be surprising, as leaf litter provides a substrate through which the vibratory signals of *Schizocosa* transmit effectively (Elias et al. 2010).

Different female maturation groups in our study population have different mate preferences. Early-maturing females prefer males of the early-maturing nonornamented morphotype, and late-maturing females prefer males of the late-maturing ornamented phenotype. In addition, our information theoretic analysis suggests that females tend to choose mates from the same substrate that they were collected from themselves. Past work in this population found that females prefer mates with the same morphotypes as their fathers and recognized that this preference could result in assortative mating (Fowler-Finn et al. 2015). Our results suggest that assortative mating may be further promoted by allochrony and differences in habitat use between groups.

Results from our study, in combination with those from previous work, lead us to a new hypothesis: the Oxford *Schizocosa* population is not a single panmictic population but rather an incipient species pair with assortative mating mediated by a combination of allochrony, habitat use, and female mate choice. The divergence of ornamented and nonornamented males in the Oxford *Schizocosa* population is likely to be a separate evolutionary event from the divergence of *S. ocreata* and *S. rovneri* in the Ohio Valley. Genetic data show that both ornamented and nonornamented spiders in the Oxford population are more closely related to *S. rovneri* than to *S. ocreata* (Hebets and Vink 2007; Fowler-Finn et al. 2015). This means that the ornamented and nonornamented morphs in Oxford are not simply sister species of *S. ocreata* and *S. rovneri*, respectively. Moreover, the directions of divergence in morphology and maturation time in the Oxford and Ohio Valley population pairs do not coincide. In Oxford ornamented morphs mature later than nonornamented morphs, but in the Ohio Valley ornamented *S. ocreata* mature earlier than nonornamented *S. rovneri* (Stratton 1984). However, this latter evidence must be taken with caution, as maturation times can evolve rapidly under selection (Haugen 2000; van Wijk et al. 2013).

In summary, the patterns we uncovered suggest that a variety of mechanisms contribute to assortative mating in a population of *Schizocosa* wolf spiders with two distinct male morphotypes. Our results show that the population includes two female forms, early and late maturing, that are strongly associated with nonornamented and ornamented males, respectively. Given the lack of genetic differentiation between the two male morphotypes (Hebets and Vink 2007; Fowler-Finn et al. 2015), much of the extensive research on this population (Hebets and Vink 2007; Fowler-Finn and Hebets 2011*a*, 2011*b*; Fowler-Finn et al. 2013, 2015; Pesek et al. 2013; Deng et al. 2014) has assumed that it is a single panmictic group. Our results provide a new context in which studies of this population should be conducted and interpreted.

How—and indeed whether—speciation can occur in the absence of complete geographic isolation has been a motivating question in evolutionary biology for more than 100 years (Mayr 1963; Coyne and Orr 2004; Fitzpatrick et al. 2008). Much of what we now know comes from the intensive study of a few clades with replicated recent or ongoing speciation events (e.g., three-spined sticklebacks [Schluter and Mcphail 1992; Schluter 1994], rift lake cichlids [Allender et al. 2003], and Darwin's finches [Grant and Grant 2014]). Our results provide new evidence for an ongoing speciation event in a population of *Schizocosa* wolf spiders. The pattern of morphological divergence in this population is similar to that found in other *Schizocosa* species pairs, suggesting that other speciation events in the clade may have involved similar mechanisms. We believe this genus can be a valuable addition to the set of systems in which speciation is commonly studied. Future work on the phylogeny and phylogeography of the genus will help us to better understand the cause and extent of its diversification.

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Literature Cited

Allender, C. J., O. Seehausen, M. E. Knight, G. F. Turner, and N. Maclean. 2003. Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. Proceedings of the National Academy of Sciences of the USA 100:14074–14079.

Anderson, D. R., and K. P. Burnham. 2002. Avoiding pitfalls when using information-theoretic methods. Journal of Wildlife Management 66:912–918.

Bell, R. D., and J. A. Roberts. 2017. Trail-following behavior by males of the wolf spider, *Schizocosa ocreata* (Hentz). Journal of Ethology 35:29–36.

Bien, J., J. Taylor, and R. Tibshirani. 2013. A lasso for hierarchical interactions. Annals of Statistics 41:1111–1141.

Bolnick, D. I., and B. M. Fitzpatrick. 2007. Sympatric speciation: models and empirical evidence. Annual Review of Ecology, Evolution, and Systematics 38:459–487.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York.

———. 2004. Multimodel inference—understanding AIC and BIC in model selection. Sociological Methods and Research 33:261–304.

Candolin, U. 1997. Predation risk affects courtship and attractiveness of competing threespine stickleback males. Behavioral Ecology and Sociobiology 41:81–87.

Clark, D. L., J. A. Roberts, and G. W. Uetz. 2012. Eavesdropping and signal matching in visual courtship displays of spiders. Biology Letters 8:375–378.

Clark, D. L., C. K. Zeeff, G. Sabovodny, A. Hollenberg, J. A. Roberts, and G. W. Uetz. 2015. The role of social experience in eavesdropping by male wolf spiders (Lycosidae). Animal Behaviour 106:89–97.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer, Sunderland, MA.

Coyne, J. A., and T. D. Price. 2000. Little evidence for sympatric speciation in island birds. Evolution 54:2166–2171.

Darwin, C. 1859. On the origin of species. J. Murray, London.

Deng, B., A. Estes, B. Grieb, D. Richard, B. Hinds, and E. Hebets. 2014. A male spider's ornamentation polymorphism maintained by opposing selection with two niches. Journal of Theoretical Biology 357:103–111.

Dondale, C. D. 1977. Life histories and distribution patterns of hunting spiders (Araneida) in an Ontario meadow. Journal of Arachnology 4:73–93.

Dondale, C. D., and J. H. Redner. 1978. Revision of Nearctic wolf spider genus *Schizocosa* (Arachneida Lycosidae). Canadian Entomologist 110:143–181.

Dres, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philosophical Transactions of the Royal Society B 357:471–492.

Dukas, R. 2008. Learning decreases heterospecific courtship and mating in fruit flies. Biology Letters 4:645–647.

Elias, D. O., A. C. Mason, and E. A. Hebets. 2010. A signal-substrate match in the substrate-borne component of a multimodal courtship display. Current Zoology 56:370–378.

Feder, J. L., and G. L. Bush. 1989. A field test of differential host-plant usage between two sibling species of *Rhagoletis pomonella* fruit flies (Diptera, Tephritidae) and its consequences for sympatric models of speciation. Evolution 43:1813–1819.

Feder, J. L., C. A. Chilcote, and G. L. Bush. 1989. Are the apple maggot, *Rhagoletis pomonella*, and blueberry maggot, *Rhagoletis mendax*, distinct species—implications for sympatric speciation. Entomologia Experimentalis et Applicata 51:113–123.

Fitzpatrick, B. M., J. A. Fordyce, and S. Gavrilets. 2008. What, if anything, is sympatric speciation? Journal of Evolutionary Biology 21:1452–1459.

Fowler-Finn, K. D., and E. A. Hebets. 2011*a*. The degree of response to increased predation risk corresponds to male secondary sexual traits. Behavioral Ecology 22:268–275.

———. 2011*b*. More ornamented males exhibit increased predation risk and antipredatory escapes, but not greater mortality. Ethology 117:102–114.

Fowler-Finn, K. D., and R. L. Rodríguez. 2012*a*. The evolution of experience-mediated plasticity in mate preferences. Journal of Evolutionary Biology 25:1855–1863.

———. 2012*b*. Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. Evolution 66:459–468.

Fowler-Finn, K. D., M. F. Rosenthal, and E. A. Hebets. 2013. Locomotor performance varies with adult phenotype in ornamented/non-ornamented wolf spiders. Ethology 119:570–580.

Fowler-Finn, K. D., L. Sullivan-Beckers, A. M. Runck, and E. A. Hebets. 2015. The complexities of female mate choice and male polymorphisms: elucidating the role of genetics, age, and mate-choice copying. Current Zoology 61:1015–1035.

Fraley, C., and A. E. Raftery. 2002. Model-based clustering, discriminant analysis and density estimation. Journal of the American Statistical Association 97:611–631.

Freedman, D. A. 1983. A note on screening regression equations. American Statistician 37:152–155.

Friedman, J., T. Hastie, and R. Tibshirani. 2010. Regularization paths for generalized linear models via coordinate descent. Journal of Statistical Software 33:1–22.

Friesen, V. L., A. L. Smith, E. Gomez-Diaz, M. Bolton, R. W. Furness, J. Gonzalez-Solis, and L. R. Monteiro. 2007. Sympatric speciation by allochrony in a seabird. Proceedings of the National Academy of Sciences of the USA 104:18589–18594.

Gaskett, A. C. 2007. Spider sex pheromones: emission, reception, structures, and functions. Biological Reviews 82:26–48.

Gauthey, Z., A. P. Hendry, A. Elosegi, C. Tentelier, and J. Labonne. 2016. The context dependence of assortative mating: a demonstration with conspecific salmonid populations. Journal of Evolutionary Biology 29:1827–1835.

Gavrilets, S. 2014. Models of speciation: where are we now? Journal of Heredity 105:743–755.

Gilman, R. T., K. Fowler-Finn, and E. A. Hebets. 2018. Data from: A probable case of incipient speciation in *Schizocosa* wolf spiders driven by allochrony, habitat use, and female mate choice. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.qc6176k.

Gordon, S. D., and G. W. Uetz. 2011. Multimodal communication of wolf spiders on different substrates: evidence for behavioural plasticity. Animal Behaviour 81:367–375.

Grant, P. R., and B. R. Grant. 2014. Forty years of evolution: Darwin's finches on Daphne Major Island. Princeton University Press, Princeton, NJ.

Haugen, T. O. 2000. Growth and survival effects on maturation pattern in populations of grayling with recent common ancestors. Oikos 90:107–118.

Hebets, E. A. 2003. Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. Proceedings of the National Academy of Sciences of the USA 100:13390–13395.

Hebets, E. A., G. E. Stratton, and G. L. Miller. 1996. Habitat and courtship behavior of the wolf spider *Schizocosa retrorsa* (Banks) (Araneae, Lycosidae). Journal of Arachnology 24:141–147.

Hebets, E. A., and L. Sullivan-Beckers. 2010. Mate choice and learning. Pages 389–393 *in* M. D. Breed and J. Moore, eds. Encyclopedia of animal behavior. Elsevier, Amsterdam.

Hebets, E. A., and C. J. Vink. 2007. Experience leads to preference: experienced females prefer brush-legged males in a population of syntopic wolf spiders. Behavioral Ecology 18:1010–1020.

Hebets, E. A., C. J. Vink, L. Sullivan-Beckers, and M. F. Rosenthal. 2013. The dominance of seismic signaling and selection for signal complexity in *Schizocosa* multimodal courtship displays. Behavioral Ecology and Sociobiology 67:1483–1498.

Herberstein, M. E., and E. Hebets. 2013. Behaviour: why are spiders good models for research? Pages 230–251 *in* D. Penney, ed. Spider research in the 21st century: trends and perspectives. Siri Scientific, Manchester.

Heuschele, J., M. Mannerla, P. Gienapp, and U. Candolin. 2009. Environment-dependent use of mate choice cues in sticklebacks. Behavioral Ecology 20:1223–1227.

Johns, J. L., J. A. Roberts, D. L. Clark, and G. W. Uetz. 2009. Love bites: male fang use during coercive mating in wolf spiders. Behavioral Ecology and Sociobiology 64:13–18.

Johnson, J. B., and A. L. Basolo. 2003. Predator exposure alters female mate choice in the green swordtail. Behavioral Ecology 14:619–625.

Kasumovic, M. M., and M. C. B. Andrade. 2006. Male development tracks rapidly shifting sexual versus natural selection pressures. Current Biology 16:R242–R243.

Kilmer, J. T., K. D. Fowler-Finn, D. A. Gray, G. Höbel, D. Rebar, M. S. Reichert, and R. L. Rodríguez. 2017. Describing mate preference functions and other function-valued traits. Journal of Evolutionary Biology 30:1658–1673.

Kokko, H., and P. Monaghan. 2001. Predicting the direction of sexual selection. Ecology Letters 4:159–165.

Kozak, G. M., and J. W. Boughman. 2009. Learned conspecific mate preference in a species pair of sticklebacks. Behavioral Ecology 20:1282–1288.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, MA.

Miller, C. W., and E. I. Svensson. 2014. Sexual selection in complex environments. Annual Review of Entomology 59:427–445.

Montgomery, T. H. 1903. Studies of the habits of spiders, particularly those of mating spiders. Proceedings of the National Academy of Sciences of the USA 55:59–149.

Moore, P. J., and A. J. Moore. 2001. Reproductive aging and mating: the ticking of the biological clock in female cockroaches. Proceedings of the National Academy of Sciences of the USA 98:9171–9176.

Nash, S., and M. Domjan. 1991. Learning to discriminate the sex of conspecifics in male Japanese quail (*Coturnix coturnix japonica*): tests of "biological constraints." Journal of Experimental Psychology 17:342–353.

Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford.

Pesek, M. F., E. A. Hebets, L. Sullivan-Beckers, and K. D. Fowler-Finn. 2013. Foreleg ornaments do not hinder foraging success in brush-legged wolf spiders. Journal of Insect Behavior 26:837–849.

Roberts, J. A., and G. W. Uetz. 2005. Information content of female chemical signals in the wolf spider, *Schizocosa ocreata*: male discrimination of reproductive state and receptivity. Animal Behaviour 70:217–223.

Romer, U., B. Engelking, and W. Beisenherz. 2014. Genetically determined mate choice can be influenced by learning in *Apistogramma cacatuoides* HOEDEMAN, 1951 (Teleostei, Cichlidae). Vertebrate Zoology 64:199–206.

Rundus, A. S., R. D. Santer, and E. A. Hebets. 2010. Multimodal courtship efficacy of *Schizocosa retrorsa* wolf spiders: implications of an additional signal modality. Behavioral Ecology 21:701–707.

Rutledge, J. M., and G. W. Uetz. 2014. Juvenile experience and adult female mating preferences in two closely related *Schizocosa* species. Journal of Arachnology 42:170–177.

Ryan, M. J., and W. Wilczynski. 1988. Coevolution of sender and receiver—effect on local mate preference in cricket frogs. Science 240:1786–1788.

Scheffer, S. J., G. W. Uetz, and G. E. Stratton. 1996. Sexual selection, male morphology, and the efficacy of courtship signalling in two wolf spiders (Araneae: Lycosidae). Behavioral Ecology and Sociobiology 38:17–23.

Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. Science 266:798–801.

———. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.

Schluter, D., and J. D. Mcphail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.

Slagsvold, T. 2004. Cross-fostering of pied flycatchers (*Ficedula hypoleuca*) to heterospecific hosts in the wild: a study of sexual imprinting. Behaviour 141:1079–1102.

Slagsvold, T., B. T. Hansen, L. E. Johannessen, and J. T. Lifjeld. 2002. Mate choice and imprinting in birds studied by cross-fostering in the wild. Proceedings of the Royal Society B 269:1449–1455.

Stoffer, B., and G. W. Uetz. 2015*a*. The effects of social experience with varying male availability on female mate preferences in a wolf spider. Behavioral Ecology and Sociobiology 69:927–937.

———. 2015*b*. Experience matters: the effects of the social environment on mate choice plasticity in a wolf spider. Integrative and Comparative Biology 55:E180.

———. 2016*a*. Social experience affects female mate preferences for a visual trait in a wolf spider. Behavioral Ecology 27:252–261.

———. 2016*b*. Tuft size matters: the effects of adult visual social experience on female mate preferences in a wolf spider. Behavioral Ecology and Sociobiology 70:2211–2221.

Stratton, G. E. 1984. Differences in maturation rates of *Schizocosa ocreata*, *Schizocosa rovneri*, their F_1 and F_2 hybrids and backcross progeny (Araneae: Lycosidae). Bulletin of the British Arachnological Society 6:193–199.

———. 1991. A new species of wolf spider, *Schizocosa stridulans* (Araneae, Lycosidae). Journal of Arachnology 19:29–39.

———. 1997. A new species of *Schizocosa* from the southeastern USA (Araneae, Lycosidae). Journal of Arachnology 25:84–92.

———. 2005. Evolution of ornamentation and courtship behavior in *Schizocosa*: insights from a phylogeny based on morphology (Araneae, Lycosidae). Journal of Arachnology 33:347–376.

Stratton, G. E., and G. W. Uetz. 1981. Acoustic communication and reproductive isolation in two species of wolf spiders. Science 214: 575–577.

———. 1986. The inheritance of courtship behavior and its role as a reproductive isolating mechanism in two species of *Schizocosa* wolf spiders (Araneae; Lycosidae). Evolution 40:129–141.

Sullivan-Beckers, L., and E. A. Hebets. 2014. Tactical adjustment of signalling leads to increased mating success and survival. Animal Behaviour 93:111–117.

Taylor, P. W., J. A. Roberts, and G. W. Uetz. 2005. Flexibility in the multi-modal courtship of a wolf spider, *Schizocosa ocreata*. Journal of Ethology 23:71–75.

Tibshirani, R. 1996. Regression shrinkage and selection via the Lasso. Journal of the Royal Statistical Society B 58:267–288.

Uetz, G. W. 2000. Signals and multi-modal signalling in spider communication. Pages 387–405 *in* Y. Espmark, T. Amundsen, and G. Rosenqvist, eds. Animal signals: signalling and signal design in animal communication. Tapir, Trondheim.

Uetz, G. W., and G. Denterlein. 1979. Courtship behavior, habitat, and reproductive isolation in *Schizocosa rovneri* Uetz and Dondale (Araneae: Lycosidae). Journal of Arachnology 7:121–128.

Uetz, G. W., and C. D. Dondale. 1979. New wolf spider in the genus *Schizocosa* (Araneae, Lycosidae) from Illinois. Journal of Arachnology 7:86–88.

Uetz, G. W., and S. Norton. 2007. Preference for male traits in female wolf spiders varies with the choice of available males, female age and reproductive state. Behavioral Ecology and Sociobiology 61:631–641.

Uetz, G. W., R. Papke, and B. Kilinc. 2002. Influence of feeding regime on body size, body condition and a male secondary sexual character in *Schizocosa ocreata* wolf spiders (Araneae, Lycosidae): condition-dependence in a visual signaling trait. Journal of Arachnology 30: 461–469.

Uhl, G., and D. O. Elias. 2011. Communication. Pages 127–189 *in* M. E. Herberstein, ed. Spider behaviour: flexibility and versatility. Cambridge University Press, Cambridge.

Vaccaro, R., G. W. Uetz, and J. A. Roberts. 2010. Courtship and mating behavior of the wolf spider *Schizocosa bilineata* (Araneae: Lycosidae). Journal of Arachnology 38:452–459.

van Wijk, S. J., M. I. Taylor, S. Creer, C. Dreyer, F. M. Rodrígues, I. W. Ramnarine, C. van Oosterhout, and G. R. Carvalho. 2013. Experimental harvesting of fish populations drives genetically based shifts in body size and maturation. Frontiers in Ecology and the Environment 11:181–187.

Verzijden, M. N., J. K. Abbott, A. C. von Philipsborn, and V. Loeschcke. 2015. Male *Drosophila melanogaster* learn to prefer an arbitrary trait associated with female mating status. Current Zoology 61:1036–1042.

Verzijden, M. N., R. E. M. Korthof, and C. ten Cate. 2008. Females learn from mothers and males learn from others: the effect of mother and siblings on the development of female mate preferences and male aggression biases in Lake Victoria cichlids, genus *Mbipia*. Behavioral Ecology and Sociobiology 62:1359–1368.

Watson, P. J. 1986. Transmission of a female sex pheromone thwarted by males in the spider *Linyphia litigiosa* (Linyphiidae). Science 233: 219–221.

Widenfalk, L. A., A. Malmstrom, M. P. Berg, and J. Bengtsson. 2016. Small-scale *Collembola* community composition in a pine forest soil—overdispersion in functional traits indicates the importance of species interactions. Soil Biology and Biochemistry 103:52–62.

Wiens, J. J., and M. J. Donoghue. 2004. Historical biogeography, ecology and species richness. Trends in Ecology and Evolution 19:639–644.

Wilgers, D. J., A. C. Nicholas, D. H. Reed, G. E. Stratton, and E. A. Hebets. 2009. Condition-dependent alternative mating tactics in a sexually cannibalistic wolf spider. Behavioral Ecology 20:891–900.

World Spider Catalog. 2017. World spider catalog. Natural History Museum, Bern.

Xie, M. G., and K. Singh. 2013. Confidence distribution, the frequentist distribution estimator of a parameter: a review. International Statistical Review 81:3–39.

References Cited Only in the Online Appendixes

Beerli, P. 1998. Estimation of migration rates and population sizes in geographically structured populations. Pages 39–54 *in* G. Carvalho, ed. Advances in molecular ecology. NATO Science Series A: Life Sciences. IOS, Amsterdam.

Li, W. H. 1976. Effect of migration on genetic distance. American Naturalist 110:841–847.

Norton, S., and G. W. Uetz. 2005. Mating frequency in *Schizocosa ocreata* (Hentz) wolf spiders: evidence for a mating system with female monandry and male polygyny. Journal of Arachnology 33:16–24.

Whitlock, M. C., and D. E. McCauley. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. Heredity 82:117–125.

Wise, D. H., and B. Cheng. 1999. Impact of intraguild predators on survival of a forest-floor wolf spider. Oecologia 121:129–137.

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Appendix from R. T. Gilman et al., "A Probable Case of Incipient Speciation in *Schizocosa* Wolf Spiders Driven by Allochrony, Habitat Use, and Female Mate Choice" (Am. Nat., vol. 192, no. 3, p. 332)

Estimating the Strength of Reproductive Isolation in the Oxford *Schizocosa* Population

Overview

Analysis of our empirical data suggests that the Oxford *Schizocosa* population is composed of two distinct maturation groups: an early group composed of early-maturing females and nonornamented males, and a late group composed of late-maturing females and ornamented males. These maturation groups are partially reproductively isolated by a combination of allochrony, habitat use, and female mate choice. In this appendix, we estimate the strength of prezygotic reproductive isolation between the maturation groups. To do this, we used a system of ordinary differential equations to track the expected populations of males and females from each maturation group seeking mates in each habitat type over the course of a mating season. We assumed that females choose mates from among males that are active at the same time and in the same habitat that they are. Females have preferred mate phenotypes, and they accept or reject males they encounter according to their preferences and preference strengths. We tracked the proportions of females from each group that choose males from their own group and from the other group to estimate the prezygotic reproductive isolation between groups. We obtained parameter values for this analysis from our empirical data and, when necessary, from the literature.

The Model

Let $F_{ij}(t)$ be the proportion of all females in mating group i that are active in the mating pool of habitat j at time t. When $i = 1$ the female is from the early group, and when $i = 2$ she is from the late group. When $j = 1$ the female is in the leaf litter habitat, and when $j = 2$ she is in the rocky habitat. Females enter the mating pool as they reach sexual maturity. In nature, most female *Schizocosa* mate only once (Norton and Uetz 2005), and therefore we assume that females leave the mating pool as they mate. We have no data on movement between habitats in our system. For simplicity, we assume that females have selected their preferred habitat by sexual maturity and that they remain in that habitat until they mate. Thus,

$$\frac{dF_{ij}(t)}{dt} = \frac{h_{ij}}{\sqrt{2\pi\sigma_{fi}^2}}\exp\left(\frac{-(\mu_{fi} - t)^2}{2\sigma_{fi}^2}\right) - r(M_{ij}(t) - a_i M_{kj}(t))F_{ij}(t). \quad \text{(A1)}$$

The first additive term in equation (A1) captures entry into the mating pool as females reach sexual maturity. In this term, h_{ij} is the probability that a female in group i matures in habitat j, μ_{fi} is the mean maturation date of females in group i, and σ_{fi}^2 is the variance in the maturation date of females in group i. The second additive term in equation (A1) captures exit from the mating pool as females mate. Here, $M_{ij}(t)$ is the density of males from group i in habitat j at time t, k indicates the maturation group that is not i, and a_i is the probability that a female in group i accepts a male from group k. The parameter r controls the mating rate in the population and is defined to include both the encounter rate and the rate at which females accept potential mates from their own maturation group. Thus, the acceptance rate a_i for members of the opposite group is measured relative to the acceptance rate for members of the female's own group.

Males enter the mating pool as they reach sexual maturity. Male *Schizocosa* can mate multiply (Norton and Uetz 2005). Therefore, we assume that males remain in the mating pool after mating and leave the mating pool when they die. As with females, we assume that males have selected their preferred habitat by sexual maturity and that they remain in that habitat thereafter. Thus,

$$\frac{dM_{ij}(t)}{dt} = \frac{D_{ij}}{\sqrt{2\pi\sigma_{mi}^2}} \exp\left(\frac{-(\mu_{mi} - t)^2}{2\sigma_{mi}^2}\right) - vM_{ij}(t). \tag{A2}$$

The first additive term in equation (A2) captures entry into the mating pool at sexual maturity, and the second additive term captures exit from the mating pool at death. The parameter D_{ij} is the total density of males in group i that mature in habitat j during the season, μ_{mi} is the mean maturation date of males in group i, σ_{mi}^2 is the variance in the maturation date of males in group i, and v is the male death rate.

Following from equation (A1), the rate at which females from group i accept mates from group k is

$$\frac{dX_{ik}(t)}{dt} = ra_i \sum_j M_{kj}(t) F_{ij}(t), \tag{A3}$$

and the proportion of females from group i that choose mates from group k is $X_{ik}(\infty)$. We solve for $X_{ik}(\infty)$ numerically.

Assigning Parameter Values

Female habitat use $\{h_{11}, h_{12}, h_{21}, h_{22}\} = \{0.6913, 0.4932, 0.3087, 0.5068\}$. We estimated the probability that females from each maturation group occupy each habitat type from our empirical data. The probability that we would have collected a female from maturation group i in the leaf litter habitat depends on the probability that females from maturation group i occupy leaf litter as well as on the intensity with which we sampled the leaf litter relative to the rocky habitat. In particular, let η_{i1} be the probability that a female from maturation group i in our data was collected from the leaf litter habitat, and let s be the intensity of sampling in the leaf litter habitat relative to sampling in the rocky habitat. Then the true probability that a female from maturation group i occupies the leaf litter habitat is

$$h_{i1} = \frac{\eta_{i1}}{s + (1 - s)\eta_{i1}}. \tag{A4}$$

If we knew the maturation group of each female in our data set, then η_{i1} would be the proportion of females from maturation group i collected in leaf litter. However, we cannot assign females to maturation groups by morphology, so we do not know the maturation group of each female with certainty. Instead, we obtained the probability that each female belongs to the early or late maturation group by applying cluster analysis to the dates on which females reached sexual maturity (see "Methods"). Let z_x be the probability that female x in our data set belongs to the early-maturation group. Then the probability that we would have found female x on leaf litter is

$$p_x = z_x\eta_{11} + (1 - z_x)\eta_{21}. \tag{A5}$$

Let $c_x \in \{0, 1\}$ be an indicator variable, where $c_x = 1$ if female x was collected from the leaf litter habitat and $c_x = 0$ if she was collected from the rocky habitat. Then the likelihood of the observed data for female x given parameters η_{11} and η_{21} is

$$\mathcal{L}(c_x|\eta_{11}, \eta_{21}) = (1 - c_x) + (2c_x - 1)p_x, \tag{A6}$$

and the log likelihood of the observed data set is

$$L_\eta = \sum_x \log(\mathcal{L}(c_x|\eta_{11}, \eta_{21})), \tag{A7}$$

where the summation runs over all females in the data set. We found the values of η_{11} and η_{21} that maximize L_η using the fminsearch function in Matlab, and we used these values to calculate h_{11} and h_{21} following equation (A4). Then $h_{i2} = 1 - h_{i1}$. Initially we assumed that sampling effort in each habitat was proportional to the area occupied by that habitat (i.e., $s = 1$), but we consider the effect of biased sampling effort below.

Female maturation time $\{\mu_{f1}, \mu_{f2,}, \sigma_{f1}^2, \sigma_{f2}^2\} = \{30.09, 48.78, 14.28, 46.19\}$. We estimated the parameter values for female maturation time from cluster analysis of our empirical data (see "Methods").

Female mate choosiness $\{a_1, a_2\} = \{0.3510, 0.1406\}$. We estimated the probability that females from each maturation group accept courting males from the opposite maturation group from our empirical data. To do this, we needed a model of mate choice. For simplicity, we assumed that each female evaluates potential mates successively and accepts or rejects each male on the basis of her preference and his phenotype, independent of any other males that may

be present in the population. Let α_{i1} be the probability that a female from maturation group i chooses an early group male in a two-choice mating trial, given that (i) the female chooses a mate in trial i and (ii) trial i includes one male from each maturation group. Then the rate at which females from maturation group i accept mates from the opposite group relative to the rate at which they accept mates from their own group is

$$a_i = (1 - \alpha_{i1})/\alpha_{i1}. \tag{A8}$$

Let z_x be the probability that a female x in our two-choice mating trials belongs to the early maturation group. If female x chooses a mate, the probability that she chooses a male from the early maturation group is

$$u_x = z_x\alpha_{11} + (1 - z_x)\alpha_{21}. \tag{A9}$$

Let $b_x \in \{0, 1\}$ be an indicator variable, where $b_x = 1$ if female x chose an early-group male and $b_x = 0$ if female x chose a late-group male. Then the likelihood of the observed data for female x given parameters α_{11} and α_{21} is

$$\mathcal{L}(b_x|\alpha_{11}, \alpha_{21}) = (1 - b_x) + (2b_x - 1)u_x, \tag{A10}$$

and the log likelihood of the observed data set is

$$L_\alpha = \sum_x \log(\mathcal{L}(b_x|\alpha_{11}, \alpha_{21})), \tag{A11}$$

where the summation runs over all females that chose mates in the mating trials. We found the values of α_{11} and α_{21} that maximize L_α using the fminsearch function in Matlab, and we used these values to calculate a_1 and a_2 following equation (A8).

Mating rate $\{r\} = \{0.04\}$. The parameter r in our model captures both the rate at which females encounter males and the rate at which females accept mates from their own maturation group. We cannot estimate r from our empirical data, and to our knowledge there are no data in the literature that would allow us to estimate this parameter accurately. However, we expect that the mating rate in nature is high. There can be more than 100 spiders per square meter in our study system (Fowler-Finn and Hebets 2011*b*) and male *Schizocosa* actively seek females (Norton and Uetz 2005), so it is likely that females encounter multiple potential mates per day. Moreover, in other *Schizocosa* populations more than 80% of females accept the first conspecific male they encounter (Norton and Uetz 2005). Initially, we set $r = 0.04$, which means that females encounter approximately two males per day when males are at their peak density. Below, we show that changing our assumptions about r has little effect on the predictions of our analysis.

Male density $\{D_{11}, D_{12}, D_{21}, D_{22}\} = \{52, 27, 41, 72\}$. We estimated the relative density of males in our study system from our empirical data. Let δ_{ij} be the observed density of males from maturation group i and habitat j in our empirical data. Then the true relative densities of males from maturation group i in the leaf litter and rocky habitats will be $D_{i1} = \delta_{i1}/s$ and $D_{i2} = \delta_{i2}$, where s is the intensity of sampling in the leafy habitat relative to the rocky habitat. Note that we have scaled the overall encounter rate between males and females using r, so it is sufficient to estimate relative densities rather than absolute densities here. Initially we assumed that sampling effort in each habitat was proportional to the area occupied by that habitat (i.e., $s = 1$), but we consider the effect of biased sampling effort below.

Male maturation time $\{\mu_{m1}, \mu_{m2}, \sigma^2_{m1}, \sigma^2_{m2}\} = \{29.87, 38.63, 79.24, 63.25\}$. We estimated the parameter values for male maturation directly from our empirical data.

Male mortality $\{v\} = \{0.033\}$. We used data collected by Wise and Chen (1999) on a mixed population of *S. ocreata* and *S. stridulans* to estimate mortality rates in natural *Schizocosa* populations. Wise and Chen (1999) recorded a survivorship rate of 0.34 over a period of ~32 days, which implies a daily mortality rate of ~0.033 d^{-1}. Wise and Chen (1999) studied juveniles, but mortality in adult males may be as high or higher. For example, adult males are subject to sexual cannibalism rates as high as 14% (Norton and Uetz 2005). A mortality rate of 0.033 d^{-1} is consistent with the short adult male life span reported by Uetz and Denterlein (1979). Below we show that changing our estimate of v has little effect on the predictions of our analysis.

Results and Discussion

Using the parameter values derived above, we estimate that females from the early and late maturation groups accept mates from the opposite group with probabilities 0.14 and 0.09, respectively (table A1). Figure A1 shows the densities of males and the proportions of females from each maturation group in the mating pool in each habitat type over time

(*A–D*) and the proportion of females from each mating group that have chosen males from their own group and from the other group over time (*E*, *F*).

Some of the parameter values we obtained were heuristic or inferred from the literature on other *Schizocosa* populations and so may not be accurate for our system. In particular, Deng et al. (2014) estimate that rocky habitat may comprise only 5%–15% of the total habitat at the Oxford study site. In our study, we aimed to collect equal numbers of spiders from the leaf litter and rocky habitats, and no attempt was made to standardize sampling intensity across habitat type. Therefore, the rocky habitat may have been sampled up to 10 times more intensively than the leaf litter habitat. Thus, we repeated the analysis above with $s = 0.1$. We estimated the male death rate in our population using data from a different system. Therefore, we repeated our analysis with male death rates three times higher and one-third as high as our default estimate. Finally, we set r in our model so that females would encounter approximately two males per day when males are at peak density. In nature, encounter rates may be higher or lower than this estimate. Therefore, we repeated our analysis assuming that females encounter one male every 2 days or 10 males per day. In each case, we estimated that the probability with which females from each maturation group choose males from the opposite maturation group is between 0.07 and 0.18 (table A1).

The strength of prezygotic reproductive isolation that we estimate here is consistent with the low levels of genetic divergence that have been observed in the Oxford *Schizocosa* population. Fowler-Finn et al. (2015) reported an F_{ST} of 0.009 between the two male morphotypes. For two populations, $F_{ST} \approx 1/(1 + 16N_e m)$, where m is the rate of gene flow from each population to the other and N_e is the effective population size (Li 1976; but for caveats see Beerli 1998; Whitlock and McCauley 1999). Thus, if $m = 0.07$, an effective population size of ~100 individuals would be large enough to prevent F_{ST} from rising above the observed level. Given that densities in this population can be >100 m^{-2}, it is plausible and even probable that the effective size of the Oxford *Schizocosa* population is at least this large.

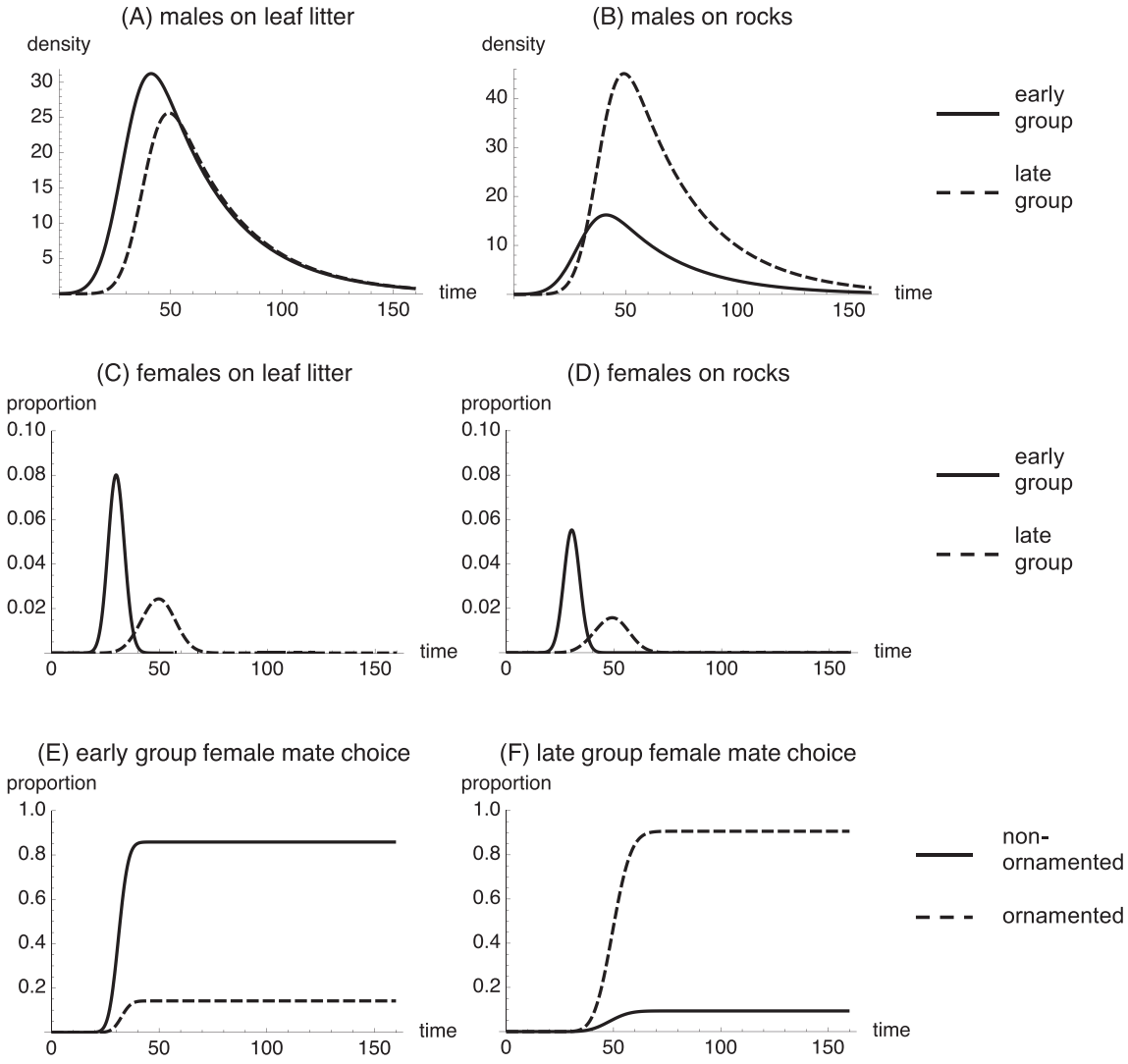

Figure A1: Population trajectories over the course of the season under the default parameter set. Shown are the density of males from each maturation group in the leaf litter (*A*) and rocky (*B*) habitat types, the proportion of the total females from each maturation group active in the mating pools in the leaf litter (*C*) and rocky (*D*) habitat types, and the proportion of all females from the early (*E*) and late (*F*) maturation groups that have chosen mates from the same or from the other maturation group.

Table A1: Estimated proportion of females from each maturation group that chose males from the opposite group under the default parameter values estimated from the data and under other plausible parameter values

Parameter value	Early group	Late group
Default	.14	.09
$s \rightarrow .1$	.09	.14
$v \rightarrow .01$	.13	.11
$v \rightarrow .1$	.17	.07
$r \rightarrow .01$	.18	.09
$r \rightarrow .2$	.13	.10

A probable case of incipient speciation in *Schizocosa* wolf spiders driven by

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Supplementary Information

No difference in the effects of central or peripheral exposure treatments on spider maturation time or mating behavior

Spiders in our study were reared in isolation or in the central or peripheral positions of quintuples (fig. 1). We conducted preliminary analyses to determine whether spiders reared in the central position of quintuples matured or behaved differently from those reared in the peripheral positions. To do this, we studied only spiders reared in quintuples. We conducted information theoretic and lasso analyses as described in the main text, replacing the factor "reared in isolation" with "reared in the central position of quintuples." This approach identifies differences in the effect of the central and peripheral treatments using all informative data. We found no such differences (fig. S1). Therefore, we merged the central and peripheral treatments into a single class (*i.e.*, "reared with exposure to other spiders") for all subsequent analyses.

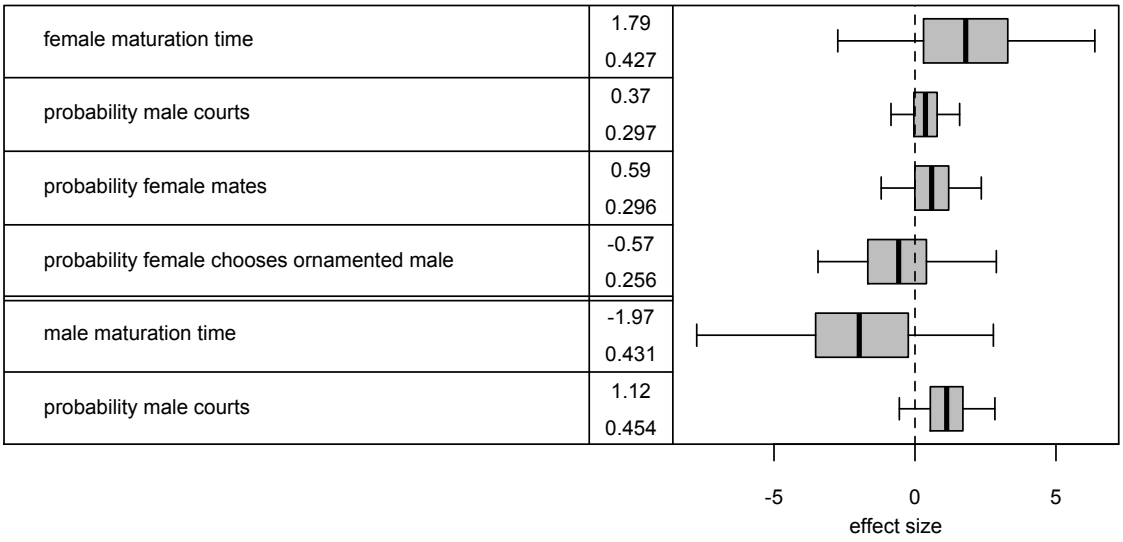

Figure S1. Effects of rearing in the central position of quintuples on spider maturation times and mating behaviours. Rows 1-4 show the effects of the female rearing condition, and rows 5-6 show the effects of the male rearing condition. In the middle cell of each row, the top number is the effect size (in days for rows 1 and 4, in log odds ratio for other rows) of the predictor, and the bottom number is the summed weight of the predictor across all fitted models. Box plots show the 50% (boxes) and 95% (whiskers) confidence intervals around the effect sizes. There is no evidence for an effect of rearing position on any response studied.

Sample and intersection sizes

Spiders in our experiments were divided into classes in many different ways. The tables below present the total sample size (top left cell), the number of spiders in each class (in parentheses, first column) and the intersections between classes (main elements of table) for the analysis of male maturation time (table S1), female maturation time (table S2), the probability of male courtship (table S3), the probability of female mating (table S4) and the probability that a female chooses an ornamented male (table S5).

Analysis of maturation time (male) **Total males: 192**	males ornamented	males reared in isolation
males ornamented (113)		
males reared in isolation (53)	26	
males collected from rocks (99)	72	33

Table S1: Analysis of male maturation time.

Analysis of maturation time (female) **Total females: 193**	females collected from rocks
females collected from rocks (89)	
females reared in isolation (43)	26

Table S2: Analysis of female maturation time.

Table S3: Analysis of male courtship.

Analysis of male courtship **Total males: 296**	females reared in isolation	competitors courted	females collected from rocks	males ornamented	trials illuminated	males used in previous trials	trials conducted on rocks	males reared in isolation	males and females from same origin	females exposed to males as juveniles	males mated in previous trials
females reared in isolation (74)		17	44	37	40	27	38	46	36	-	5
competitors courted (65)			37	10	56	6	28	34	42	16	1
females collected from rocks (144)				72	102	37	62	90	89	50	6
males ornamented (148)					111	29	72	64	88	41	6
trials illuminated (222)						36	108	104	131	58	5
males used in previous trials (72)							34	38	36	21	13
trials conducted on rocks (144)								76	78	46	4
males reared in isolation (151)									80	45	5
males and females from same origin (171)										43	3
females exposed to males as juveniles (82)											3
males mated in previous trials (13)											
males collected from rocks (158)	50	32	89	93	117	39	74	90	89	43	8

Table S4: Analysis of female mating.

Analysis of female mating **Total females: 148**	females courted	females reared in isolation	trials conducted on rock	females collected from rocks	females exposed to males as juveniles	males mated in previous trials	males used in previous trials	females exposed to ornamented males as juveniles
females courted (87)		37	39	50	17	1	13	14
females reared in isolation (37)			19	22	-	1	12	-
trials conducted on rock (72)				31	23	1	12	18
females collected from rocks (72)					25	1	13	21
females exposed to males as juveniles (41)						1	9	29
males mated in previous trials (6)							6	0
males used in previous trials (29)								3
females exposed to ornamented males as juveniles (33)								
trials illuminated (111)	68	20	54	51	29	3	11	26

Analysis of female choice **Total females: 80**	trials in which ornamented male courted first	trials in which males courted	trials in which the ornamented male was collected from the same habitat as the female	trials conducted on rocks	females reared in isolation	females exposed to ornamented males as juveniles	females exposed to males as juveniles	trials in which the non-ornamented male was collected from the rocks	females collected from the rocks	trials in which the ornamented male was collected from the rocks	trials illuminated
trials in which ornamented male courted first (39)		39	15	14	11	7	10	17	26	27	32
trials in which males courted (61)	39		23	24	25	12	15	26	39	43	47
trials in which the ornamented male was collected from the same habitat as the female (26)	15	23		11	8	6	8	4	22	22	20
trials conducted on rocks (31)	14	24	11		11	12	14	13	19	20	22
females reared in isolation (25)	11	25	8	11		-	-	15	17	19	12
females exposed to ornamented males as juveniles (19)	7	12	6	12	-		19	9	16	11	16
females exposed to males as juveniles (24)	10	15	8	14	-	19		9	18	14	19
trials in which the non-ornamented male was collected from the rocks (32)	17	26	4	13	15	9	9		19	24	22
females collected from the rocks (49)	26	39	22	19	17	16	18	19		37	35
trials in which the ornamented male was collected from the rocks (55)	27	43	22	20	19	11	14	24	37		40
trials illuminated (58)	32	47	20	22	12	16	19	22	35	40	
trials in which the ornamented male was older (19)	9	14	5	8	5	6	7	7	11	11	13

Table S5: Analysis of female mate choice.

Predictors of male courtship when the extreme outlier is removed from the data set

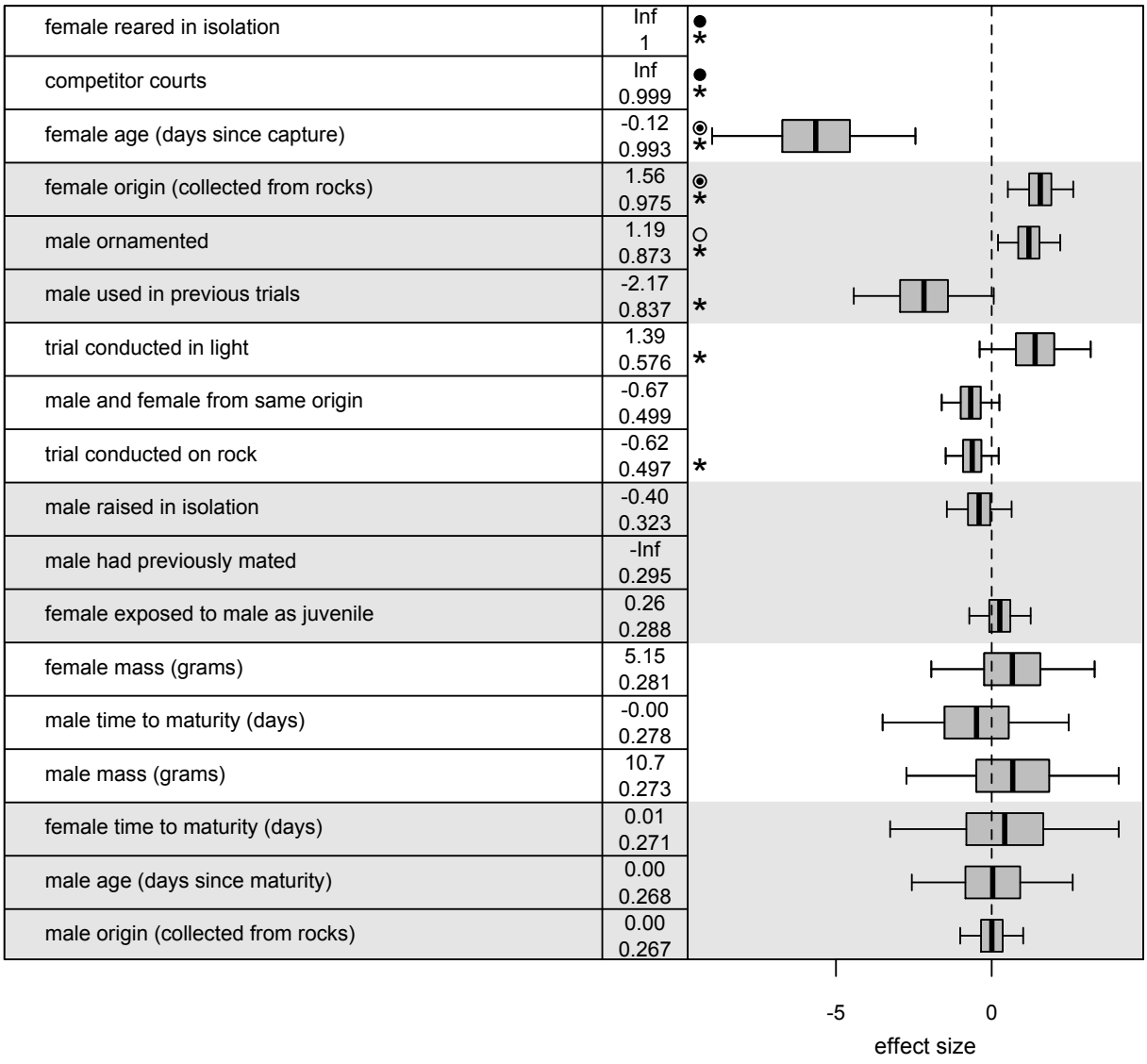

Figure S2: Predictors of male courtship when the extreme outlier is removed from the data set. In the middle cell of each row, the top number is the effect size of the predictor on the log odds ratio of male courtship, and the bottom number is the summed weight of the predictor across all fitted models. Box plots show the 50% (boxes) and 95% (whiskers) confidence intervals around the effect sizes (scaled as in fig. 4). ●, ⊙ and ○ indicate that zero falls outside of the 99.9%, 99% and 95% confidence intervals, respectively, for the effect size of the predictor. ✶ indicates that the predictor was identified as meaningful by the

lasso analysis. For three predictors, no box plots are shown. These are cases in which there is perfect separation in the data. For example, when the outlier male is excluded, every remaining male courted whenever his competitor courted. In such cases, the maximum likelihood effect size for the predictor is infinite. Nonetheless, even when a predictor is perfect, we might have little evidence for an effect of that predictor if the number of correct predictions is small. This is the case for the effect of mating history on the likelihood of male courtship (note the small summed weight of the predictor).

Female origin does not explain bimodality in days to maturity

modes	days to maturity	residuals of days to maturity regressed on female origin
1	0.0001	0.0001
2	0.9597	0.9450
3	0.0402	0.0550

Table S6: Relative likelihoods of distributions with 1, 2 or 3 modes for female days to maturity, and for the residuals of days to maturity regressed on female origin. Because the residuals of days to maturity regressed on female origin are bimodally distributed, female origin does not explain the bimodality in days to maturity.